

The best information that Applicants have been able to obtain indicates that the publication date of the Palmer reference is December 1986. This information is based on details concerning the Palmer reference which were found on a Web site maintained by Amazon.com, and which can be located at the following URL: <http://www.amazon.com>. See Attachment A, a copy of Applicants' search results based on the Palmer reference. Applicants' copy of the Palmer reference which is being provided with their Information Disclosure Statement additionally indicates that the reference was received by the University of California, San Diego, on April 29, 1987, which date does not conflict with Applicants' determination that the publication date of the Palmer reference is December 1986. Since Applicants have no evidence that the 31 base nucleotide sequence disclosed by Palmer was actually disclosed at the Proceedings of the Sixth International Symposium on Human Chlamydial Infections, Applicants will assume for purposes of the attached Declaration that the publication date of Palmer's nucleotide sequence is December 1986.

To remove Palmer as a possible prior art reference, Applicants are submitting herewith a Declaration Under 37 C.F.R. § 1.131, executed by James J. Hogan, Richard D. Smith, JoAnn Kop Dileanis and Sherrol H. McDonough, co-inventors of the subject application, which swears back of Palmer's publication date. This Declaration references and describes evidence demonstrating conception and reduction to practice by the inventors of an oligonucleotide probe which hybridizes to *Chlamydia trachomatis* rRNA in the 16S rRNA region corresponding to bases 170-230 and not to RNA from *Chlamydia psittaci*. The probe identified in this Declaration was produced by a process which included identifying a variable region present in the 16S rRNA region corresponding to bases 170-230 of *Chlamydia trachomatis* and *Chlamydia psittaci* and substantially maximizing complementarity of the probe to the variable region present in *Chlamydia trachomatis* rRNA and substantially minimizing complementarity of the probe to the variable region present in *Chlamydia psittaci* rRNA. The conception and reduction to practice activities evidenced in the Declaration took place in the United States and were completed prior to December 1986 (all dates appearing in the originals of Exhibits A, B, C and D have been redacted).

Exhibit A of the Declaration shows a comparison of DNA sequences complementary to sequences of *Chlamydia trachomatis* and *Chlamydia psittaci* contained in the 16S rRNA region corresponding to bases 170-230 of *Escherichia coli* which was performed by Ame Holden, a Research Associate supervised by Sherrol McDonough, prior to Palmer's publication date. See Exhibit A, Book No. 329, page 32. From this sequence comparison, 12 base differences between *Chlamydia trachomatis* and *Chlamydia psittaci* were identified. Under Sherrol McDonough's supervision, Ame Holden diligently submitted a sequence request form based on the sequence complementary to *Chlamydia trachomatis* 16S rRNA ("ChtA0176") to Mohammed Majlessi. See Exhibit A at page 40. Mohammed Majlessi then diligently synthesized and purified the requested sequence prior to the publication date of Palmer. See Exhibit B, Book No. 320, page 39. Optical density measurements indicated the presence of nucleic acid in the purified sample, and an autoradiograph confirmed that the synthesized sequence was 33 nucleotides in length. See Exhibit B at pages 39-40. Additionally, a trityl-off procedure was used to analyze the coupling efficiency of each of the phosphoramidites in the synthesis procedure and to further confirm that the desired sequence had been synthesized. See Exhibit B at page 39.

Mary Harper then obtained the probe and diligently labeled it with  $^{32}\text{P}$  by kinasing. See Exhibit C. The labeled probe was then transferred to Paula Roeder, who diligently used the probe for cross-reactivity testing detailed in Exhibit D prior to the publication date of Palmer. Mary Harper and Paula Roeder were working as a Staff Scientist and a Research Scientist, respectively, in assignee's Product Development group at the time their activities were performed.

Paula Roeder's cross-reactivity testing examined the specificity of the ChtA0176 probe for *Chlamydia trachomatis* RNA in the presence of RNA from *Chlamydia psittaci*. To do this, hybridization mixtures were prepared in pairs of scintillation vials, each member of each pair containing a DIBBS diluent, the ChtA0176 probe, an iodinated pan-bacterial probe, and one of the following: (i) RNA from a lysate of one of five *Chlamydia trachomatis* serotypes; (ii) RNA from a

lysate of one of three *Chlamydia psittaci* serotypes; (iii) RNA from a single *Chlamydia trachomatis* serotype; (iv) RNA from *Escherichia coli*; and (v) a negative control containing no added RNA. See Exhibit D at pages 39 and 42. After exposing the vials to identical hybridization conditions, a separation solution containing hydroxyapatite (HA) was provided to the vials and the vials were heated and centrifuged to separate out the HA. See Exhibit D at page 35. To distinguish signal from bound and unbound probe, the supernatant of each vial was decanted into another scintillation vial. This process of heating, centrifuging and decanting was repeated after resuspending the HA in each vial with a wash solution. Signal from each vial was determined in a scintillation counter.

The results of Paula Roeder's cross-reactivity study are set forth at page 41 of Exhibit D. Here, the "FP" samples correspond to vials containing the supernatants only, and the "BP" samples correspond to vials containing HA. Because HA binds to double-stranded nucleic acid, signal from the BP samples should represent bound probe and signal from the FP samples should represent probe which did not bind to nucleic acid in the hybridization mixture. Thus, the percent hybridization for any given pair of hybridization mixtures is the average of the counts per minute (cpm) values for BP over the total cpm value (BP + FP). These values are recorded at page 42 of Exhibit D and show that the ChtA0176 probe hybridized to nucleic acid from each of the five serotypes of *Chlamydia trachomatis* and not to nucleic acid belonging to *Escherichia coli* or to any of the three serotypes of *Chlamydia psittaci*. The results also show that there was no hybridization in the negative control hybridization mixtures.

To demonstrate that there was bacterial RNA in hybridization mixtures other than the negative controls, the percentage of hybridized pan-bacterial probe in each HA-containing vial was measured by Paula Roeder. See Exhibit D at pages 40 and 42. This was done by preparing a new scintillation vial containing amounts of HA and the pan-bacterial probe equivalent to those contained in each of the HA-containing vials. See Exhibit D at page 40. The counts per minute in the newly prepared vial were determined and used as a measure of the total counts per minute for the pan-

bacterial probe. CytoScint was then added to each HA-containing vial and the counts per minute were determined for each vial. The percentage of hybridization for each hybridization mixture was calculated by dividing the counts per minute in each HA-containing vial by the total counts per minute. The percentages of related pairs of hybridization mixtures were averaged and compared. *See Exhibit D at page 42.* As expected, the results indicated that bacterial RNA was present in each of the hybridization mixtures except the negative controls.

For the reasons presented above, Applicants submit that the Palmer reference has been properly sworn back of and is unavailable as a prior art reference.

#### **Conclusion**

Applicants submit that the subject application is in condition for allowance and Notice to the effect is respectfully requested.

Please charge any fees due in connection with this Supplemental Response to Deposit Account No. 07-0835 in the name of Gen-Probe Incorporated.

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SUPPLEMENTAL RESPONSE

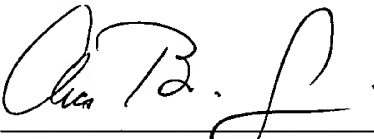
Serial No. 08/454,529  
Atty. Docket No. GP004-16.DV4

At your direction, this correspondence, and any referred to as attached, is being sent by Federal Express on the date indicated below to Examiner Ardin H. Marschel, Art Unit 1631, United States Patent and Trademark Office, 7<sup>th</sup> Floor Receptionist, 1911 South Clark Place, Crystal Mall One, Arlington, Virginia 22202.

Respectfully submitted,

Dated: June 28, 2002

By:

  
Charles B. Cappellari  
Registration No. 40,937  
Attorney for Applicants

GEN-PROBE INCORPORATED  
Patent Department  
10210 Genetic Center Drive  
San Diego, California 92121  
PH: (858) 410-8927  
FAX: (858) 410-8928

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